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The LOB-like transcription factor Mt LBD1 controls *Medicago truncatula* root architecture under salt stress

Federico D. Ariel,[1,†] Anouck Diet,[2,3,†] Martin Crespi[2,*] and Raquel L. Chan[1,*]

[1]Instituto de Agrobiotecnología del Litoral; Consejo Nacional de Investigaciones Científicas y Técnicas; Universidad Nacional del Litoral; Santa Fe, Argentina; [2]Institut des Sciences du Végétal; Centre National de la Recherche Scientifique; Gif sur Yvette; [3]Université Paris Diderot Paris 7; Les Grands Moulins; Paris, France

[†]These authors contributed equally to this work.

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Abbreviations: LR, lateral root; ABA, abscisic acid; IAA, indol-acetic acid; Mt, *Medicago truncatula*; LOB, LATERAL ORGAN BOUNDARIES; LBD, LOB-domain; HD-Zip, homeodomain-leucine zipper

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*Correspondence to: Raquel Chan and Martin Crespi;
Email: rchan@fbcb.unl.edu.ar and crespi@isv.cnrs-gif.fr

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Lateral root (LR) formation and emergence are influenced by the environment and determines the architecture of the root system in the soil. Whereas auxins appear as the main hormone controlling LR initiation, patterning and emergence, abscisic acid (ABA) is the key hormone mediating the effect of the environment on root architecture. Hormone signaling act through transcription factors (TFs) and the *Medicago truncatula* LOB-like TF LBD1 was shown to be auxin-inducible but repressed by the HD-Zip I TF MtHB1 in response to salt stress and ABA during LR formation. Here, we demonstrate that the constitutive expression of Mt *LBD1* in Medicago roots alters their global architecture when the plant is subjected to salt stress. Hence, LBD1 may control the final form of the root system in the soil environment.

To optimize their growth, plants adapt their root architecture to the environmental constraints for maximizing the availability of nutrients and water. In legumes, such as *Medicago truncatula*, the symbiotic interaction of specific Rhizobiaceae bacteria with the root may lead to the formation of new organs, the nitrogen-fixing root nodules. As legumes are major worldwide crops, it is crucial to understand how the environment influences their root growth and architecture through hormone action and signaling. Auxin controls LRs formation, regulating their initiation, primordium patterning and emergence.[1-4] The local accumulation of auxin in individual xylem pericycle cells serves as an instructive signal to select specific cells to initiate LRs.[5] Then, further divisions form the LR primordia and lead to the emergence of the LR from the primary root through breakage of the epidermal cells.[1,3] Members of the plant-specific LATERAL ORGAN BOUNDARIES (LOB) Domain gene family are characterized by a LOB domain and a predicted coiled coil structure that is reminiscent of a leucine zipper.[6] Several LBD (LOB BINDING DOMAIN) genes are linked to different aspects of root formation.[7] In monocots, the *CRL1/ARL1* and *RTCS* genes in rice and maize exert orthologous functions during shoot-borne root formation.[8-10] In dicots, the Arabidopsis genes *LBD16* and *LBD29* are involved in LR formation as their overexpression enhances this process whereas dominant repression of *LBD16* inhibits LR initiation.[11] *LBD16* and *LBD29* are directly activated by the AUXIN RESPONSE FACTORS ARF7 and ARF19 and are early auxin responsive genes.[11] Similarly, ARF1 is able to recognize the promoter region of the rice auxin-inducible gene *CRL1/ARL1,* suggesting a conserved regulatory mechanism.[7]

The response to environmental stresses, such as salt and drought stress, is mainly mediated by the hormone abscisic acid (ABA). In *Arabidopsis thaliana*, ABA regulates LR initiation and emergence through the ABI3 TF[12,13] and also represses auxin response in LR primordia. The control exerted by ABA on root branching can be relevant under stress conditions to optimize the root

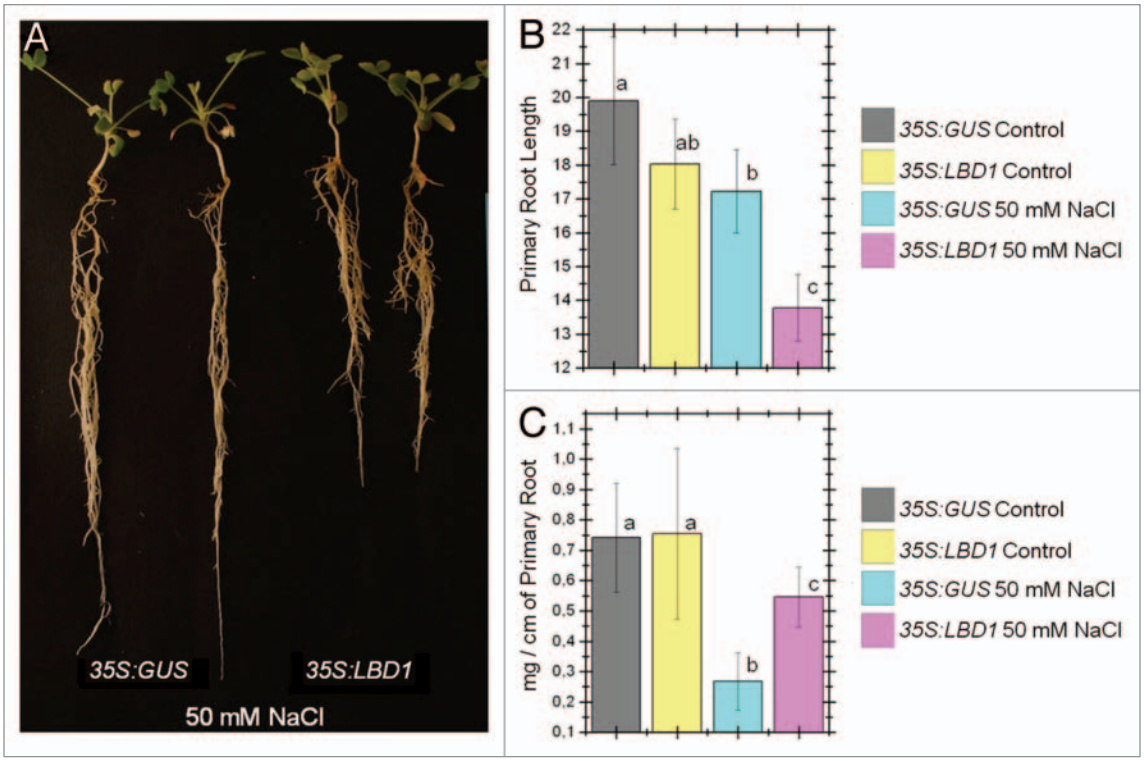

Figure 1. *M. truncatula LBD1* affects root architecture. (A) Representative examples of composite plants with roots transformed with the constructs *35S:GUS* (control) or *35S:LBD1*, grown 14 days on sand/perlite in the greenhouse and watered with poor "i" medium containing 50 mM NaCl.[17] (B) Primary root length of *35S:GUS* and *35S:LBD1* plants in control conditions and in response to 50 mM NaCl. (C) Root dry weight per centimeter of primary root under control and salt stress (50 mM NaCl) conditions. In (B and C), the letters indicate mean values significantly different among groups (Kruskal-Wallis test $p < 0.05$, $n > 20$ in every case).

surface exposed to the soil environment and consequently, root growth.[4,14] In *Medicago truncatula,* the HD-Zip I TF HB1 mediates the repressive effect of salt stress and ABA on LR formation.[15] Overexpression of *HB1* in *M. truncatula* roots and the analysis of *hb1* TILLING mutants revealed that this gene regulates the emergence of LRs from the primary root. MtHB1 directly recognizes the cis-element CAATAATTG present in the LOB-like gene *LBD1* promoter. This LOB-like gene is induced by auxins during LR formation and repressed by salt stress and ABA, through MtHB1.[15]

Overexpression of *LBD1* Affects *Medicago truncatula* Root Architecture

LBD1 is transcriptionally induced by a 3 h treatment with 0.1 μM IAA in *M. truncatula* roots. Such induction was reversed to a repression when IAA was applied together with 100 mM NaCl or 100 μM ABA. Hence, we analysed the consequences of de-regulating Mt *LBD1* on the root system under control and stress conditions. To this end, we amplified the *LBD1* gene from the BAC clone AC146551 and inserted it in the binary plasmid pMF2, following the constitutive promoter CaMV 35S.[16] Medicago transgenic roots were obtained as previously described in reference 17, using the empty vector as a control. Composite plants were transferred to a perlite:sand mixture and grown in the greenhouse, irrigated with "i" medium with or without 50 mM NaCl.[15] After 14 days, plants were taken out of the soil, and the root system was analyzed by measuring the main root length and the root dry weight per cm. No significant differences in root growth or phenotype were scored for Mt *LBD1* overexpressing roots in control conditions. However, under salt stress, the *35S:LBD1* roots were significantly shorter and exhibited a higher mass per cm of main root (**Fig. 1**). Hence, these roots showed a significant change in their architecture in response to salt stress. The reduction in primary root growth was compensated by an increase in root mass likely through LR formation.

As 50 mM NaCl has a major effect on Medicago LR emergence, without significantly altering LR initiation, we think that *LBD1* overexpression promoted LR emergence. Indeed, we have recently shown that the MtHB1 TF directly represses *LBD1* during LR formation, and that this TF affects only LR emergence.[15] Hence, repression of *LBD1* leads to enhanced primary root growth and reduction of LR formation. These previous results together with the consequences of *LBD1* de-regulation demonstrates that LBD1 plays a key role in the determination of LR formation and root architecture in legumes. Future studies on LBD1 target genes may reveal how this TF determines the final form of the root system in *Medicago truncatula*. It will also be of interest to analyze the eventual conservation of this regulatory mechanism, involving the ABA-regulated Mt HB1 and the auxin-inducible Mt LBD1 transcription factors in Arabidopsis, other dicots or even in monocots.

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